Differences in serum IgG structure in health and rheumatoid disease

Circular dichroism studies

P. M. JOHNSON, J. WATKINS, P. M. SCOPES,* AND B. M. TRACEY*
From the Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berkshire, and the Department of Chemistry, Westfield College, Hampstead, London NW3 7ST*

There is evidence for the presence of structural anomalies in the serum IgG of patients with rheumatoid arthritis. Such evidence derives from papain hydrolysis studies (Watkins, Unger, and Mahon, 1970) and catabolic studies of rheumatoid IgG in man (Levy, Barnett, MacDonald, and Klinenberg, 1970; Watkins and Swannell, 1972, 1973) and mouse (Watkins, Turner, and Roberts, 1972). Further evidence for the existence of structural anomalies in these proteins can be obtained from circular dichroism (CD), a spectropolarimetric technique widely used for studying conformational changes in biological macromolecules (Beychok, 1966). In a preliminary communication, two of us have already reported some physicochemical evidence for structural anomalies in IgG based on CD studies (Johnston and Watkins, 1974) and we now report a broader survey of the serum IgG of patients suffering from a variety of connective tissue disorders. The CD spectra of these samples are compared with those of healthy individuals and of patients suffering from active tuberculous disease, and the significance of our observations is discussed.

Materials and methods

Immunoglobulin G was isolated from the sera of 23 individuals by column chromatography on DEAE-cellulose* with 0.01 mol/l. phosphate buffer, pH 6.2. The survey included seven patients suffering from seropositive rheumatoid arthritis who had active disease as judged by the criteria of the American Rheumatism Association (1959), two patients suffering from seropositive rheumatoid arthritis whose disease was judged to be inactive, five patients suffering from seronegative rheumatoid arthritis, two patients suffering from Still's disease, and two patients with an early diagnosis of systemic lupus erythematosus. None of these patients were receiving gold or corticosteroid therapy. Also included as controls were two patients suffering from chronic tuberculous disease and three healthy individuals who were members of the hospital staff. Immunoglobulin G was similarly isolated from the synovial fluid of two patients suffering from seropositive rheumatoid arthritis.

All IgG preparations were shown to be free from other serum proteins by electrophoretic and serological techniques. They possessed no plasmin activity as evaluated by the fibrin plate method (Marsh and Arocha-Pinango, 1972). Papain and peptic fragments, obtained from Kabi Cohn II digests, were a gift from Dr. M. W. Turner of the Institute of Child Health, London.

Measurements of the circular dichroism spectra were made on a Cary 61 recording spectropolarimeter at 27°C. All samples, previously dialysed against 0.01 mol/l. phosphate buffer, pH 7.4, were passed through a sterile 0.45 μm millipore filter immediately before CD analysis. Protein concentrations and cell path lengths were such that the absorbance at 280 nm did not exceed 1.5 absorbance unit. The concentrations were determined spectrophotometrically at 280 nm, immediately after the CD spectra had been obtained, using extinction coefficients of E1% = 14.0 for IgG and 15.0 for fragments (Doi and Jirgensons, 1970). A mean residue weight of 109 was assumed in all calculations (Doi and Jirgensons, 1970). Data are expressed as mean residue ellipticity in deg. cm²/dmol (θ).

Results

The CD spectra of Fab and F(ab')2 fragments are shown in Fig. 1. The most marked variations in the spectra occur at 275–280 nm. Since the only difference between Fab and F(ab')2 molecules is the presence of the inter-heavy chain disulphide bridging region in F(ab')2, the so-called 'hinge' region, it seems probable that transitions occurring within one or more amino acids in this region are responsible for the negative circular dichroism of F(ab')2 compared with Fab between 275 and 280 nm. Slight differences in the CD spectra at longer wavelengths may result from transitions within tryptophan residues (Dorrington and Smith, 1972). Further evidence for the implication of the hinge region in the transition near 280 nm comes from comparison of the CD spectra of intact
Differences in serum IgG structure

Kabi human IgG before and after breakage of the disulphide bonds by reduction with dithioerythritol (reduction and alkylation were carried out essentially as described by Litman, Good, Frommel, and Rosenberg, 1970). The spectra for these reduced and alkylated proteins show a marked reduction in the negative dichroism at 280 nm as compared with the intact molecule, and were very similar to that of the rheumatoid IgG shown in Fig. 2.

The CD spectrum of serum IgG from healthy volunteers was found to be remarkably reproducible, despite the heterogeneous nature of IgG. It was surprising that not only the relative intensities of the discrete peaks but also much of their finer structure appeared consistent. The negative transition at 280 nm appeared to be characteristic of normal human IgG and was a constant feature of commercial samples of this protein. Thus we conclude that there is little significant variation in the overall conformation of the molecular population of the serum IgG in healthy individuals.

The CD spectra of the serum IgG of two healthy patients are compared with those of two patients suffering from active seropositive rheumatoid arthritis in Figs 2 and 3. Isolation of the serum IgG and determination of the CD spectra for each pair, normal and rheumatoid, were conducted consecutively and under parallel conditions. The spectra differ significantly only in two regions, near 280 nm and 294 nm. Rheumatoid IgG appears to have a decreased negative maximum at 280 nm and an increased positive maximum at 294 nm. Both of these effects appear to be enhanced in IgG isolated from rheumatoid sera that has been 'aged', or better, partially denatured by multiple thawing and freezing, as shown in Fig. 3. We consider that the differences in the spectra at 280 nm are more significant than those at 294 nm, not only because of the evidence for the implication of
the hinge region in this transition, but also because a separate study of monoclonal IgG shows that the value of the positive maximum at 294 nm is variable in a manner that may suggest nonspecific interaction phenomena (Johnson, Scopes, Tracey, and Watkins, 1974).

The results of a study of the CD band at 280 nm in serum IgG isolated from patients suffering from various connective tissue disorders and from further control individuals are summarized in the Table; the negative transition at 280 nm is characterized as strong, slight, or none, according to its intensity. Patients with rheumatoid disease predominantly show little or no discrete negative absorption in the CD spectra of their serum IgG at 280 nm, compared to the controls. In fact, only one of the 16 rheumatoid IgG samples, from a patient with seronegative rheumatoid arthritis, showed a marked spectral peak at 280 nm; this patient had marked paraproteinaemia and may be atypical. In addition, IgG isolated from the synovial fluid of patients suffering from active seropositive rheumatoid arthritis appears similar in CD characteristics to the serum IgG isolated from patients with the same diagnosis. In this small group there was no significant difference in the intensity of the 280 nm transition between the seropositive and seronegative rheumatoid patients, despite the known better prognosis of the latter. However, it is possible that a study of a greater number of patients might reveal differences.

It is known that degradative changes can occasionally be caused by the proteolytic action of traces of contaminating plasmin in isolated and stored IgG, and presumably such activity would proceed at a different rate for each sample. Since our results, with regard to an anomaly in the hinge region of the serum IgG, could be explained by plasmin activity, we have taken care to ensure that the IgG preparations used in this study contained no plasminogen assayed by double immunodiffusion against anti-plasminogen serum, and also possessed no plasmin activity assayed on the fibrin plate method. Hence, it appears that patients with rheumatoid disease have a structural anomaly in some, if not all, of their serum IgG molecules.

Table  Magnitude of transition at 280 nm in circular dichroic spectra of IgG for controls and for patients suffering from a variety of connective tissue disorders

<table>
<thead>
<tr>
<th>Diagnosis (no. of cases)</th>
<th>Source of IgG</th>
<th>Incidence of 280 nm CD peak intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individuals (3)</td>
<td>Serum</td>
<td>Strong 3/3</td>
</tr>
<tr>
<td>Tuberculosis (2)</td>
<td>Serum</td>
<td>Slight 0/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None 0/3</td>
</tr>
<tr>
<td>Rheumatoid arthritis (16)</td>
<td>Serum</td>
<td>Strong 0/7</td>
</tr>
<tr>
<td>(a) Active seropositive</td>
<td>Synovial fluid</td>
<td>Slight 0/2</td>
</tr>
<tr>
<td>(b) Active seronegative</td>
<td>Serum</td>
<td>None 0/2</td>
</tr>
<tr>
<td>(c) Inactive seropositive</td>
<td>Serum</td>
<td>Strong 1/5*</td>
</tr>
<tr>
<td>(d) Seronegative</td>
<td>Serum</td>
<td>Slight 1/5</td>
</tr>
<tr>
<td>Still’s disease (2)</td>
<td>Serum</td>
<td>None 1/2</td>
</tr>
<tr>
<td>Systemic lupus erythematosus (2)</td>
<td>Serum</td>
<td>Slight 1/2</td>
</tr>
</tbody>
</table>

* Patient showed marked paraproteinaemia, IgG1L-type.
Discussion

Circular dichroism has its origin in the absorption bands of optically active compounds and measures the unequal absorption of right and left circularly polarized light (Beychok, 1966). The main feature of the CD spectra of human IgG is a large negative dichroism maximum at 217 nm which has been shown to be characteristic of the $\beta$-structure of the protein (Cathou, Kulczycki, and Haber, 1968); this feature appears relatively invariant. At longer wavelengths the spectra arise from the overlap of discrete absorptions due to transitions occurring in tyrosine, tryptophan, and to a lesser extent phenylalanine side chain residues, and also from the asymmetric environment of disulphide linkages. Although it is now possible to study this area of the spectrum in detail, the exact assignment of the bands is not known.

The serum IgG of patients with rheumatoid disease has been shown to have a reduced negative maximum in the CD spectra at 280 nm as compared with the serum IgG of healthy individuals. This region of the spectrum may be associated with transitions occurring within the hinge region of the IgG molecule on the basis of the changes in the CD spectra on mild reduction and alkylation of IgG and also a comparison of the CD spectra of Fab and F(ab')2 fragments. Furthermore, two of the seropositive rheumatoid patients referred to in this study had significantly increased rates of serum IgG catabolism (Watkins and Swannell, 1972), suggesting that these proteins were modified structurally. This evidence for a structural anomaly in the hinge region of rheumatoid IgG supports the hypothesis suggested by our earlier work on the papain hydrolysis of rheumatoid IgG and the catabolism of rheumatoid IgG in man and mouse.

Natvig, Gaarder, and Turner (1972) have previously implicated a series of amino acid residues in the $pF_6$ region of human IgG as potentially antigenic in the rheumatoid process, and have suggested the presence of other such sites in the Fc fragment. Our results suggest that the hinge region may also be a potentially antigenic site.

The possible nature of the structural anomaly is less clear. There could be three explanations:

(a) a genetic primary sequential difference;
(b) the binding of another molecule, of unknown nature, at or near the hinge region which acts as a stimulus to a structural change;
(c) an altered heterogeneity of the IgG molecular population in terms of either conformation or of IgG subclass production.

There is little evidence of a direct familial incidence of rheumatoid arthritis (Cobb, Schull, Harburg, and Kasl, 1969), and consequently a genetic difference in the primary structure of the IgG of rheumatoid patients, or the potential rheumatoid patient, seems unlikely.

Alternatives (b) and (c) are more difficult to resolve, particularly as they are to some extent complementary. Our serological tests indicated an absence of IgA, IgM, transferrin, caeruloplasmin, $\beta$-lipoprotein, orosomucoid, and all other major serum proteins from the IgG preparations used for CD studies. We have also tested for the possible effect of specific antiglobulins, themselves IgG immunoglobulins, by comparing the CD spectra of IgG samples isolated both from total serum and from serum following affinity chromatography (on insolubilized human IgG) to remove antiglobulins. There was no significant difference in the CD spectra for either normal IgG or seropositive rheumatoid IgG after the specific absorption of the antiglobulins. This is not surprising since the reported concentrations of IgG antiglobulins in the sera of rheumatoid patients appears to be too low to alter significantly the CD spectra (Torrigiani and Roitt, 1967; Iliter and Turner, 1973).

In contrast, a direct conformational change of some of the IgG molecules might expose antigenic sites on the molecule and lead to autoantibody production. Such a conformational change in the IgG molecule may arise, for example, by amide-imide rearrangements or changes in the carbohydrate moiety attachments, and is entirely consistent with a stable primary amino acid sequence. Alternatively, our results might be explained by a change in the heterogeneity of the IgG molecular population. This could be induced by a preference in either anabolism or catabolism and may correlate with the increased incidence of paraproteinaemia observed for patients suffering from rheumatoid disease (Zawadzki and Benedek, 1969). Clonal preference in anabolism could reflect the production of antibody of low affinity for an antigen or autoantigen, whereas preferential catabolism could reflect a fault of recognition, which might result in the formation of immune complexes. The latter have a pathogenic role in such diseases as rheumatoid arthritis and systemic lupus erythematosus. A genetic bias of IgG subclass proportions and their individual conformations is an intriguing possibility, but an accurate study is hindered by the limited specificity of available 'subclass-specific' antisera.

In conclusion, there is both physicochemical and physiological evidence for a structural anomaly in the serum IgG of patients with rheumatoid disease. We suggest this may arise either by intramolecular rearrangements or as a result of selective metabolism.

Summary

We have studied the circular dichroism (CD) spectra of serum IgG isolated from eighteen individuals suffering from a variety of connective tissue dis-
orders and compared them with the spectra obtained from five control individuals. Differences between these two groups were noted in the region near 280 nm indicating some differences in structure of the serum IgG of rheumatoid patients. From a study of enzymically and chemically derived fragments of IgG, the hinge region of the IgG molecule has been implicated in this structural difference. The possible nature and pathological significance of such a structural difference is discussed.

References

AMERICAN RHEUMATISM ASSOCIATION (1959) Ann. rheum. Dis., 18, 49 (Diagnostic criteria for rheumatoid arthritis, 1958 revision)

BEYCHOK, S. (1966) Science, 154, 1288 (The circular dichroism of biological macromolecules)

CATHOU, R. E., KULCZYCKI, A., and HABER, E. (1968) Biochemistry, 7, 3958 (Structural features of γ-immunoglobulin, antibody, and their fragments: circular dichroism studies)


DOI, E., and JIRGENSONS, B. (1970) Biochemistry, 9, 1066 (Circular dichroism studies on the acid denaturation of γ-immunoglobulin G and its fragments)

DORRINGTON, K. J., and SMITH, B. R. (1972) Biochim. biophys. Acta, 263, 70 (Conformational changes accompanying the dissociation and association of immunoglobulin G subunits)


WATKINS, J., and SWANENELL, A. J. (1972) Ibid., 31, 218 (Enhanced catabolic rate and a structural anomaly in the serum IgG of RA patients)

——, —— (1973) Ibid., 32, 247 (Catabolism of human serum IgG in health, rheumatoid arthritis and active tuberculous disease)


Differences in serum IgG structure in health and rheumatoid disease. Circular dichroism studies.
P M Johnson, J Watkins, P M Scopes and B M Tracey

doi: 10.1136/ard.33.4.366

Updated information and services can be found at:
http://ard.bmj.com/content/33/4/366.citation

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/